

Safety Data Sheet

Chlorozotocin

Division of Safety
National Institutes
of Health



WARNING!

THIS COMPOUND IS TOXIC, CARCINOGENIC, AND MUTAGENIC. IT IS READILY ABSORBED THROUGH THE INTESTINAL TRACT. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND COLD WATER. AVOID WASHING WITH SOLVENTS. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

IN CASE OF FIRE, USE WATER-BASED OR DRY CHEMICAL EXTINGUISHER.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, DRINK MILK OR WATER. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. SEE CASTEGNARO ET AL. (1985) FOR DETAILS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

Introductory Note

There are only few data in the literature concerning physical, chemical, and some biological properties of CLZ.^A Where such data do not exist, the information published here is based on analogies with STR and with other β -chloroethyl compounds such as CCNU.

^AAbbreviation used in this Data Sheet. Other abbreviations used are:
STR=Streptozocin; CCNU=1-(2-chloroethyl)-3-cyclohexyl-nitrosourea.

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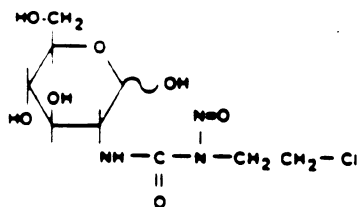
A. Background

Chlorozotocin (CLZ), a compound of glucose and N-(2-chloroethyl)-N-nitroso-urea, is an ivory-colored crystalline compound, stable in dry form and in solution at slightly acid pH, unstable in strong acid and alkali, and soluble in water and lower molecular weight alcohols. It is toxic in all mammalian species tested, carcinogenic and mutagenic. CLZ has been used experimentally as an antineoplastic in the treatment of mouse L1210 leukemia and of rat mammary tumors. It is noteworthy that CLZ does not show the diabetogenic properties of its close relative, STR. Toxic side effects are mainly confined to liver and kidney, with little or no effect on the hematopoietic system.

General reviews are: Johnston and Montgomery (1986); Mitchell and Schein (1986).

B. Chemical and Physical Data

1. Chemical Abstract No.: 54749-90-5.
2. Synonyms: 2-[[[(2-chloroethyl)nitrosoamino]-carbonyl] amino]-2-deoxy-D-glucose; CHLZ; CLZ; CZT; DCNU; D-glucose, 2-[[[(2-chloroethyl)-nitrosoamino]-carbonyl] amino]-2-deoxy;^B NSC-178248; urea, 1(2-chloroethyl)-3-(D-glucopyranos-2-yl)-1-nitroso-.
3. Chemical structure and molecular weight:



C₉H₁₆ClN₃O₇; 313.7

4. Density: No data.
5. Optical activity: There are no data on optical activity and mutarotation due to optical isomerism at carbon atom 1 of the glucose ring. In analogy with STR, this undoubtedly exists but has not been investigated beyond a mention of α and β anomers (Johnston et al., 1975).

^BChemical Abstracts name, used for listing in 9th Decennial Index and subsequently.

Absorption spectroscopy: CLZ has a strong ultraviolet absorption maximum at 248 nm, and weaker maxima at 231 and 367 nm. The linear absorption range is 10-300 µg/ml. There is also fluorescence ($\lambda_{\text{ex}} = 325$, $\lambda_{\text{em}} = 440$ nm) with a range of $0.4\text{--}3.2 \text{ ml} \times 10^{-6}$ (Pavlik et al., 1983). Infrared absorption data have been published (Johnston et al., 1975).

Volatility: No data, may be regarded as essentially non-volatile.

Solubility: CLZ is highly soluble in water. There are no quantitative data but it may be assumed that it is somewhat soluble in lower molecular weight alcohols and insoluble in nonpolar solvents.

Description: Ivory-colored crystals.

Boiling point: No data; **melting point:** 140-141°C with decomposition (Johnston et al., 1975).

Stability: In analogy with STR and CCNU the dry powder is likely to be stable for a year or more in sealed ampoules at room or refrigerator temperatures. Solutions of CLZ show highest stability around pH 4. They are unstable at neutral pH: $t_{1/2}$ in tris buffer, pH 7.4 = 30 min (Plowman et al., 1978). Decomposition products at pH 7 or 7.4 include acetaldehyde, chloroethanol (Montgomery et al., 1975), and a number of 5-membered ring carbamate sugars (Hammer et al., 1981). This hydrolysis is accompanied by loss of ultraviolet absorption. The effect of pH and buffer composition has been studied (Chatterji et al., 1978). The scheme for decomposition may be similar to that proposed for other β -chloroethyl nitrosoureas (e.g., Colvin et al., 1976).

Chemical reactivity: The glucose moiety of CLZ is converted to the tetracetyl derivative which is also an anticarcinogen (Anderson et al., 1975) and presumably undergoes other reactions characteristic for glucose. Other reactions which are common with other β -chloroethyl nitrosoureas include interaction with DNA by alkylation (Gombar et al., 1980; Weinkam and Dolan, 1983). It is noteworthy however that, in contrast with other β -chloroethyl nitrosoureas, CLZ has little or no carbamoylating activity (Anderson et al., 1975), possibly due to competing intramolecular carbamoylation (Hammer et al., 1981) mentioned above.

Flash point: No data.

Autoignition temperature: No data.

Fire, Explosion, and Reactivity Hazard Data

1. CLZ is likely to be inactivated under conditions of fire. Fire-fighting personnel should wear protective clothing and face masks. Use water-based or dry chemical extinguishers.
2. Flammability is likely to be low.
3. Conditions contributing to instability are acid, alkali, and elevated temperatures.
4. Hazardous decomposition products under conditions of fire are likely to include hydrochloric acid and nitrogen oxides. The formation of acetaldehyde and 2-chloroethanol has been reported for the aqueous hydrolysis of CLZ and these compounds may also be decomposition products on ignition.

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The NIH Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving CLZ.

It should be emphasised that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation: Validated methods have been reported (Castegnaro et al., 1985).
2. Decontamination: Turn off equipment that could be affected by CLZ or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Consult Castegnaro et al. (1985) for details concerning decontamination of surfaces, glassware, and animal cages.

3. Disposal: It may be possible to decontaminate waste streams containing CLZ before disposal. For details, see Castegnaro et al. (1985). No waste streams containing CLZ shall be disposed of in sinks or general refuse. Surplus CLZ or chemical waste streams contaminated with CLZ shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing CLZ shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing CLZ shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with CLZ shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing CLZ shall be handled in accordance with the NIH radioactive waste disposal system.
4. Store solid CLZ in unopened vials. Avoid exposure to light and moisture. Store working quantities of CLZ and its solutions in an explosion-safe refrigerator in the work area. See B11 for further information.

Monitoring and Measurement Procedures including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data. As for STR, tissue and blood samples should be stored in ice after preparation or dilution with buffer.
2. Analysis: No methods for the analysis of CLZ have been published. (Investigations of the metabolic fate of CLZ have relied on measurement of radioactivity derived from variously labeled CLZ). It is likely that the colorimetric method (Forist, 1964), which was developed for STR, can be adapted.

Biological Effects (Animal and Human)

1. Absorption: CLZ is readily absorbed after parenteral (intravenous, intraperitoneal) injection. There are no data regarding absorption from the gastrointestinal tract.
2. Distribution and pharmacokinetics: (Note: Information discussed in this and in the subsequent section on metabolism and excretion is almost entirely based on studies with CLZ labeled in one of two positions which will be designated as

follows: a: C₁ of the glucose moiety, and b: the chloro-ethyl group. Differences in results of such studies indicate extensive breakup of the CLZ molecule in the animal body and offer no indication of retention or excretion of intact CLZ.)

Intravenously or intraperitoneally injected CLZ disappears rapidly from plasma in a biphasic mode, with $t_{1/2}$ of 14 and 146 min, in rats and dogs (Plowman et al., 1978). Similar results are found in mice (Mhatre et al., 1978). These findings were the same whether a or b-labeled CLZ were employed, indicating that not much if any metabolism takes place prior to distribution to tissues. Considerable binding of radioactivity to plasma proteins occurs, and the time course of this binding (increase after initial decline of plasma radioactivity) suggests tissue metabolism followed by rerelease of metabolites to the blood stream and subsequent protein binding.

Metabolism and excretion: In rats and dogs, 75% of the a label is found in the urine within 6 hours after administration, and 4% in the bile. For the b label these figures are 37 and 40%, again indicating metabolism of CLZ in tissues. The bile contains one major and two minor components, not as yet identified. Considerable radioactivity is found in the kidney and liver; in the latter organ activity due to label b is about five times that of label a. In vitro experiments with human bone marrow show alkylation of DNA, DNA strand breakage and cross linking, similar to the effects found with other β -chloroethyl nitrosoureas such as CCNU; in contrast with the latter, however, CLZ binds preferentially to transcriptionally inactive regions of bone marrow chromatin (Byane et al., 1984). The transport of CLZ into cells appears to differ from that of STR for which the glucose moiety is the transporting agent (Lazarus et al., 1983).

Toxic effects: The acute LD₅₀ in the rat (ip, iv) is 22-28 mg/kg which makes CLZ about 5 times more toxic than the closely related STR. In the mouse, at least, the dose-response curve appears to be quite steep, the intraperitoneal LD₁₀ and LD₉₀ being 20 and 30 mg/kg, respectively (Mhatre et al., 1978).

Toxic side effects in animals and man are similar to those encountered with STR: nausea and vomiting with little or no effect on the hematopoietic system. In the latter effect CLZ differs from other β -chloroethyl nitrosoureas (Mitchell and Schein, 1986). In dogs and monkeys, relatively high doses result in renal tubular damage and some bone marrow hyperplasia (Gralla et al., 1976; Schein et al., 1976). It should be noted that CLZ shows none of the specific effects of STR on pancreatic β cells and no diabetogenic action. It should also be

noted that CLZ has little or no carbamoylating activity, thus confirming previous impressions that this activity is not related to toxic (or carcinogenic) action of compounds in this series.

5. Carcinogenic effects: Weekly intraperitoneal treatment of rats with CLZ results in a dose-dependent incidence of malignant tumors (mesotheliomas or sarcomas of the peritoneal cavity) (Habs et al., 1979). This is the only reference to this effect, and in the absence of reports on other species or routes of administration the specificity of this effect is open to question.
6. Mutagenic and teratogenic effects: CLZ is highly mutagenic in the Ames test and against Drosophila (Zimmer and Bhuyan, 1976; Franza et al., 1980; Kortselius, 1978; Suling et al., 1983). There are no data concerning teratogenicity.

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. Skin should not be rinsed with organic solvents. Avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.
2. Ingestion: Drink plenty of water or milk. Refer for gastric lavage.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician. Consider treatment for liver or kidney involvement.

References

- Anderson, T.M., G. McMenamin, and P. Schein. 1975. Chlorozotocin, 2-[3-(2-chloroethyl)-3-nitroso ureido]-D-glucopyranose, an antitumor agent with modified bone marrow toxicity. *Cancer Res* 35:761-765.
- Byane, P., K. Tew, J. Jemionek, T. MacVittie, L. Erickson, and P. Schein. 1984. Cellular and molecular mechanisms of bone marrow sparing effects of the glucose chloroethyl nitrosourea chlorozotocin. *Blood* 63:759-767.

- Castegnaro, M., J. Adams, M.A. Armour, J. Barek, J. Benvenuto, C. Confalonieri, U. Goff, S. Ludeman, D. Reed, E.B. Sansone, and G. Telling. 1985. Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Antineoplastic Agents. IARC Scientific Publications No. 73. World Health Organization, Geneva, Switzerland.
- Chatterji, D.C., R.F. Greene, and J.F. Gallelli. 1978. Mechanisms of hydrolysis of halogenated nitrosoureas. *J Med Chem* 67:1527-1532.
- Colvin, M., R.B. Brundrett, W. Cowens, I. Jardine, and D.B. Ludlum. 1976. A chemical basis for the antitumor activity of chloroethyl nitrosoureas. *Biochem Pharmacol* 25:695-699.
- Forist, A.A. 1964. Spectrophotometric determination of streptozotocin. *Anal Chem* 36:1338-1339.
- Franza, B.R., Jr., N.S. Oeschger, M.P. Oeschger, and P.S. Schein. 1980. Mutagenic activity of nitrosourea antitumor agents. *J Natl Cancer Inst* 65:149-154.
- Gombar, C.T., W.P. Tong, and D.B. Ludlum. 1980. Mechanism of action of the nitrosoureas. IV. Reactions of bis-chloroethyl nitrosourea and chloroethyl cyclohexyl nitrosourea with deoxyribonucleic acid. *Biochem Pharmacol* 29:2639-2643.
- Gralla, E.J., R.W. Fleischman, A.M. Guarino, D.A. Cooney, and R.D. Davis. 1976. Toxicology studies with chlorozotocin (NSC 178,248) in beagle dogs and rhesus monkeys. U.S. NTIS, PB Rep 254569; *Chem Abstr* 86:65492g.
- Habs, M., G. Eisenbrand, and D. Schmahl. 1979. Carcinogenic activity in Sprague-Dawley rats of 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose (chlorozotocin). *Cancer Lett* 8:133-137.
- Hammer, C.F., R.A. Loranger, and P.S. Schein. 1981. Structures of the decomposition products of chlorozotocin: New intramolecular carbamates of 2-amino-2-deoxyhexoses. *J Org Chem* 46:1521-1531.
- Johnston, T.P., G.S. McCaleb, and J.A. Montgomery. 1975. Synthesis of chlorozotocin, the 2-chloroethyl analog of the anticancer antibiotic streptozotocin. *J Med Chem* 18:104-106.
- Johnston, T.P. and J.A. Montgomery. 1986. Relationship of structure to anticancer activity and toxicity of the nitrosoureas in animal systems. *Cancer Treat Rep* 70:13-30.

Kortselius, M.J.H. 1978. Mutagenicity of BCNU and related chloroethyl nitrosoureas in Drosophila. Mut Res 57:297-305.

Lazarus, P., J. St. Germina, M. Dufour, G. Palmer, D. Wallace, and L.C. Panasci. 1983. Comparison of the transport of chlorozotocin and CCNU in L1210 leukemia and murine bone marrow cells in vitro. Cancer Chemother Pharmacol 11:159-161.

Mhatre, R.M., D. Green, L.C. Panasci, P. Fox, P.V. Woolley, and P.S. Schein. 1978. Pharmacologic disposition of chlorozotocin in mice. Cancer Treat Rep 62:1145-1151.

Mitchell, E.P. and P.S. Schein. 1986. Contribution of nitrosoureas to cancer treatment. Cancer Treat Rep 70:31-41.

Montgomery, J.A., R. James, G.S. McCaleb, M.C. Kirk, and T.P. Johnston. 1975. Decomposition of N-(2-chloroethyl)-N-nitrosourea in aqueous media. J Med Chem 18:568-571.

Pavlik, E.J., D.E. Kenady, J.R. van Nagell, K. Keaton, M.B. Hansen, E.S. Donaldson, W.O. Griffen, and R.C. Flanigan. 1983. Properties of anticancer agents relevant to in vitro determinations of human tumor cell sensitivity. Cancer Chemother Pharmacol 11:8-15.

Plowman, J., J.V. Dingell, and R.H. Adamson. 1978. Disposition of chlorozotocin in rats and dogs. Cancer Treat Rep 62:31-44.

Schein, P.S., L. Panasci, P.V. Woolley, and T. Anderson. 1976. Pharmacology of chlorozotocin (NSC-178248), a new nitrosourea antitumor agent. Cancer Treat Rep 60:801-805.

Suling, W.J., L.S. Rice, and W.M. Shannon. 1983. Increased mutagenicity of chloroethyl nitrosoureas in the presence of rat liver S9 microsome mixture. J Natl Cancer Inst 70:767-769.

Weinkam, R.J. and M.E. Dolan. 1983. An anylsis of 1-(2-chloroethyl)-1-nitrosourea activity at the cellular level. J Med Chem 26:1656-1659.

Zimmer, D.M. and B.K. Bhuyan. 1976. Mutagenicity of streptozotocin and several other nitrosourea compounds in Salmonella typhimurium. Mut Res 40:281-288.